Current Research on Chemical Composition, Analytical Methods, and Quality Standards of Amorphophallus konjac

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Research Article

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Abstract

Amorphophallus konjac, a traditional Chinese medicine, possesses remarkable anti-tumor properties, exerting effects such as scattered stasis detumescence, detoxification, and pain relief. This medicinal herb is frequently employed in contemporary clinical settings to treat malignant tumors. However, the cultivation and processing of this herb lack standardisation, making it challenging to control its quality effectively. The current quality control standards for Amorphophallus sinensis Belval are not uniform, the detection methods are relatively limited, and the specificity is comparatively weak. Consequently, these standards fail to meet authenticity requirements, identify counterfeit products, and evaluate the quality of Amorphophallus sinensis Belval herbal materials and related medications. To enhance clinical efficacy, it is essential to review the existing analysis and identification methods as well as the quality control standards. This review aims to serve as a valuable resource for enhancing and optimising the quality evaluation system for Amorphophallus sinensis Belval, facilitating improved clinical outcomes.

Background

Amorphophallus konjac, also known as Konjac, refers to the tuber of perennial herbaceous plants. Various species, including Dongchuan konjac (also known as East China konjac), warstem Konjac, and southern snake stick, can be utilised as medicinal materials. It possesses a bitter, cold, and toxic taste and exhibits detoxification, analgesic, expectorant, and stasis-dispelling effects. Modern medicine is frequently employed for treating liver, gastric, colorectal, and other malignant tumors, which is considered an effective anti-tumor medicine[1]. Additionally, it is known to reduce blood fat, promote weight loss, and exhibit anti-fatty liver properties. Although *Amorphophallus konjac* was first documented in the Zhejiang Pesticide Annals[2] and is commonly used in folk medicine to treat tumors, it is not included in the "Chinese Pharmacopoeia". In some regions, Konjac is commonly used as a decoction piece in traditional Chinese medicine to treat tumors[3]. However, in practical applications, challenges arise due to confusion about the medicinal base and the similar morphology of the medicinal plants, resulting in inconsistent and variable product quality. Moreover, local quality control standards are inconsistent, and numerous deficiencies significantly impact the effectiveness and safety of these medicinal materials. Consequently, a comprehensive evaluation of the existing relevant standards for *Amorphophallus konjac* is necessary to improve and optimise the quality control standards, ensuring the efficacy and safety of Snake Valley products.

Chemical composition of Amorphophallus konjac

Research on the chemical composition of *Amorphophallus konjac* is relatively limited, with fewer reported studies. However, researchers have utilised various analytical techniques such as high-performance liquid chromatography, thin layer chromatography, gas chromatography, and solvent extraction methods to investigate its chemical constituents. The analysis revealed that *Amorphophallus konjac* primarily contains polysaccharides, proteins, amino acids, alkaloids, and trace elements. Notably, konjac glucomannan is often recognised as the primary active component with anti-tumor properties. In a study conducted by Chang Zhongfei et al.[4], qualitative analysis of the six extracts from *Amorphophallus konjac* indicated that the ethyl acetate fraction contained alkaloids, volatile oil, organic acids, flavonoids, coumarin, lactone, and other components. Additionally, the petroleum ether fraction contained volatile oil.

1.1 Polysaccharide components

Polysaccharide components found in Konjac, including *Amorphophallus konjac*, comprise glucomannan, starch, cellulose, glucose, and mannose[5]. Among these components, konjac gluganan (Konjac glucomannan, KGM) constitutes the majority, accounting for over 50% of the content[6]. KGM is a low-calorie and low-fat edible plant fiber with various beneficial effects, such as promoting intestinal activity[7], aiding in weight loss, suppressing tumor growth, enhancing immunity, anti-aging properties, and improving blood sugar levels [8]. Glucomannan found in Konjac is recognised as an ideal dietary fiber, and Konjac products have been listed among the "top ten health foods" by the World Health Organization[9]. These products find extensive application in the food, chemical, pharmaceutical, and bioengineering industries. In research conducted by Cui Xi et al.[10], it was discovered that Konjac does not contain KGM, whereas *Amorphophallus konjac* does. Maeda and colleagues[11] proposed that KGM is a reserve polysaccharide isolated from the konjac tuber. It comprises β-D-mannose (Man) and β-D-glucose (Glc) in a molar ratio of 1.6:1, primarily connected by β-1,4 bonds with some branching structures present. In addition to the analysis conducted on the KGM structure, researchers also investigated the hydrolysis products of methylated samples using GC-MS. This analysis confirmed that D-mannose and D-glucose residues in KGM possess branch points at carbon 3 (C3) within the 1,4-linked chain structure. Zhang and colleagues[12] discovered that konjac glucomannan is comprised of β-D-mannose (Man) and β-D-glucose (Glc) in a molar ratio of 1.6:1 or 1.4:1. This ratio may vary depending on the specific konjac varieties. At the same time, the relative molecular weight remains consistent. The fundamental structure of KGM is depicted in Fig. 1. According to Meng Fanbing et al. [13], natural glucomannan can be classified into two forms: α (amorphous) and β (crystalline). Xu Wei et al.[14] conducted a study using a mouse model of Lewis lung cancer. They evaluated the impact of KGM on nuclear transcription factors using the enzyme-linked immunosorbent assay (ELISA). The findings suggested that KGM has the potential to inhibit tumor angiogenesis, subsequently suppressing tumor proliferation and metastasis, ultimately leading to the inhibition of malignant tumors. Fu Hongbing[15] conducted a study to determine the glucomannan content using a spectrophotometer. The results indicated that medium-sized Konjac plants cultivated under light conditions exhibited the highest KGM content, measuring 3.311 mg/mL.

Starch is one of the major components of Snake Six Valley's polysaccharides. Guo Sheng et al.[16] found that the glucan content in the tubers exhibited a negative correlation with starch. Additionally, Konjac does not naturally contain glucomannan but can contain up to 70% starch. Wang Yao et al.[17] suggested that the extraction process conditions for konjac glucomannan can effectively preserve its structure and original physical and chemical properties. Acid hydrolysis of fresh konjac tubers revealed a measured starch content of 4.62% (± 0.63%).

1.2 Protein and amino acid composition

According to Wang Chengjun et al.[18], *Amorphophallus konjac* contains approximately 9.7% crude protein and 16 amino acids. The protein content generally hovers around 7.8%. Among the amino acids present, seven are considered essential, including phenylalanine, methionine, lysine, valine, and leucine[22]. Zhou Qiwu et al.[19] found that the protein content in five different konjac samples ranged from 7.86–4.16%, which was relatively higher than reported in previous literature studies. According to Sun Tianwei[20], the crude protein content in three konjac samples, particularly white Konjac, was approximately 11%, significantly higher than the other two varieties. However, the free amino acid content in all three samples was approximately 1%. Additionally, Konjac processing by-products contain up to 20% protein and about 1% amino acids. Chen Yongbo[21] proposed a specific correlation between Konjac's disease resistance and the content of water-soluble amino acids in its leaves. Based on the experimental findings, it has been observed that the content and type of water-soluble amino acids in healthy flower konjac leaves are lower compared to those in leaves affected by soft rot. This suggests that the application of fertilisers rich in serine, threonine, alanine, proline, and low or no phenylalanine and tryptophan, as well as selenium-rich fertilisers, may effectively enhance the disease resistance of Konjac.

1.3 Alkaloids

Alkaloids are a class of organic compounds known for their potent bioactive pharmacological effects. They are predominantly nitrogen-containing heterocyclic compounds and are a significant source of modern drugs[22]. In Konjac, alkaloids have been identified, with imidazoles and amides being the primary types[23]. These alkaloids play a crucial role in the medicinal properties of Konjac. Long deqing[24], through preliminary isolation of konjac alkaloids, deduced the presence of 5-methyl-2-substituted-1H-imidazole or 5-methyl-4 substituted-1H-imidazole derivatives. According to Ma Jun[25], *Amorphophallus konjac* contains alkaloids in the range of I%~2%. It also contains a higher concentration of calcium oxalate crystals, which can be toxic. Therefore, appropriate processing is necessary to attenuate the toxicity before it can be used as a medicinal substance. Pang Jie et al.[26] detected over 20 different types of alkaloids in Konjac. After extracting and purifying the alkaloids from Snake Six Valley, one of the samples was identified as a 5-methimidazole derivative, as illustrated in Fig. 2. According to Sun Tianwei[20], it is believed that alkaloid content can vary among different varieties of Konjac and even within different parts of the same Konjac plant. For example, the alkaloid content is generally lowest in the underground stem, while the top bud tends to have the highest alkaloid content. During the processing of Konjac, a significant amount of konjac fly powder is often generated. To prevent wastage, the processed scraps are analysed and examined. It has been suggested that these scraps may contain purine alkaloids, imidazole alkaloids, and colchicine alkaloids.

1.4 Inorganic composition

According to Wang Chengjun et al.[18], *Amorphophallus konjac* is believed to contain trace elements such as magnesium, iron, calcium, potassium, sodium, manganese, and copper. Yan Ruiwen et al.[27] found that *Amorphophallus konjac* possesses numerous elements essential for the human body. Through plasma atomic emission spectra (ICP-AES) analysis, it was observed that the content of elements such as K, Ca, and Mg was relatively high, followed by Na, Fe, Mn, and Zn. Dong Kun[28] utilised inductively coupled plasma mass spectrometry (ICP-MS) to examine Konjac and found significant amounts of elements such as Na, Mg, Fe, K, Ca, Al, and Zn. Small amounts of Be, V, Sb, Ba, TI, Pb, Th, and U were also detected, while Cr and Ag were not found. The study also suggested that the soil in which Konjac is grown is a crucial factor affecting the content of trace elements. Different soil conditions in various regions can lead to variations in the types and concentrations of trace elements in Konjac.

1.5 Ceramide

Ceramide, belonging to the N-acyl sphingolipids, is composed of a long-chain base called sphingosine and a fatty acid. Its basic structure is depicted in Fig. 3. The content of ceramide in Konjac is approximately 1.5%-5.0% [29]. Different forms of sphingosine can give rise to different types of ceramides. Sphingosine can be categorised into three types: sphingosine (SPH), dihydrosphingosine (DHS), and phytosphingosine (PHS), as shown in Fig. 4[30]. Ceramides can be broadly classified into five categories[31], as depicted in Fig. 5. It has been discovered that ceramide plays a role in regulating biological growth and exhibits anti-tumor properties. Ceramide has been recognised for its diverse functions, including its ability to prevent cardiovascular disease, delay inflammation, slow down the aging process, and moisturise skin. These properties have made ceramide widely used in various industries, such as daily chemical products, medicine, and the food industry.

Through literature review, it has been discovered that the ceramide content in Konjac is ten times higher than corn, rice bran, and wheat[30]. Zhu Lian Lian[32] demonstrated that microbial fermentation effectively extracts ceramide from konjac fly powder. Zhou Xiuxiu[29] determined the ceramide content in konjac samples using efficient liquid phase-evaporative light scattering detection (HPLC-ELSD). The study found that the ceramide content in konjac skin was higher than in konjac powder. Furthermore, the ceramide content can vary among different parts of the Konjac plant and different varieties. Mass spectrometry analysis identified a specific ceramide compound with an m/z value of 410.34, a relative molecular weight of 409.34, and a molecular composition of $C_{23}H_{43}N_3O_3$. This compound is 5-amino-4-hydroxy-2-oxazole-2'-hexadecyl-ceramide, as depicted in Fig. 6. In the research conducted by Ding Yaping[33], ceramide was utilised and extracted from Konjax using liquid extraction and column chromatography. The extraction processes resulted in an approximate yield of 0.05%. The molecular formula of the extracted ceramide was determined to be $C_{24}H_{47}NO_3$, as illustrated in Fig. 7. In a study by Luo Chunhong[34], supercritical carbon dioxide fluid extraction technology was employed to extract ceramide from Konjac. The optimised extraction conditions included an extraction pressure of MPa, a 55% ethanol solution, and a separation pressure of 10 MPa. Under these conditions, the extraction yielded approximately 1.73 mg/g of ceramide, with an efficiency of 0.24%.

1.6 Flavonoids

Flavonoids, specifically flavones, are a group of natural antioxidants widely utilised in the food and pharmaceutical industries. Structural type, substituents, binding mode, polymerisation, etc, influence their pharmacological activities. The core structure of flavonoids, known as the parent core structure (C6-C3-C6), is depicted in Fig. 8. Flavonoids are abundant in various plant organs, exhibiting significant variation in species and content. Ding Yaping[31] detected that flower konjac tubers contain minimal or no flavonoids, whereas other parts of Konjac or different Konjac varieties contain flavonoids. Zhou Xiuxiu[29], through qualitative analysis using color reactions, suggested that Konjac does contain flavonoids, although the exact types cannot be determined. Based on the experimental results, it is speculated that Konjac powder may contain flavonols and isoflavones, while Konjac skin may contain dihydroflavonoids and dihydroflavonols.

In the study conducted by Tian Mengnan et al.[35]], the optimisation of the extraction process for total flavonoids from Konjac was investigated using the methanol-ultrasound-assisted extraction method. The total flavonoid content was determined, and the study examined the influence of ultrasound time, ultrasonic temperature, methanol concentration, and liquid ratio on the extraction yield. Through research conducted by Xiao Longyun[36], the extraction rate of flavonoids from Konjac reached 2.13% by exploring the impact of various factors on the extraction process. Furthermore, antioxidant experiments demonstrated that Konjac exhibits a strong antioxidant capacity, highlighting its potential health benefits. Chen Bailing[37] conducted a color reaction experiment to detect flavonoids in Konjac, including flavonones, isoflavones, and chalcones. The content of flavonoids was determined to be 3.487 mg/g using aluminum nitrate and ultraviolet spectrophotometric methods.

1.7 Volatile oil

According to Zhou Xiuxiu[29], the volatile oil in Konjac was extracted using various solvents such as methanol, ethanol, ethyl acetate, and acetonitrile. The analysis revealed the presence of 23 active components, predominantly alkane substances. Among these components, 11 were common in all four extraction solvents, including 5-isobutyl-nonane and L-ascorbic acid 2,6-hexadecanoic acid. Ethyl acetate extraction yielded 17 active ingredients and 10 common components, with a relative content of up to 85%. In the study conducted by Tian Mengnan[35], the volatile oil in two batches of Konjac samples was extracted using the methanol-ultrasonic extraction method. It was found that the types and relative content of volatile oil differed between konjac powder and konjac skin.

1.8 Other ingredients

In the research conducted by Ma Jun et al.[25]], several compounds were isolated and identified after processing Konjac, including betulinic acid, β-sitosterol, squalene, xylose, carotene, riboflavin, thiamine, and ascorbic acid. The underground tuber of *Amorphophallus konjac* also contains tannins, while fresh Konjac contains polyphenol oxidase[38]. Konjac leaves contain trimethylamine, while the inflorescence is rich in vitamin B_1[39]. Fresh Konjac contains eight vitamins, including five B vitamins (V_B family), as well as vitamins A, D_3 and E[40]. Regarding the dietary fiber content, Zhou Qiwu et al.[19] reported crude fiber content of 4.7% and a range of 0.06–0.54% for different Konjac samples. Wang Yao et al.[17] detected a dietary fiber content of 12.05% (± 1.04%) in fresh konjac tubers using the enzyme-weight method.

Analytical methods

2.1 Trait identification

According to the Anhui Province traditional Chinese medicine decoction pieces processing standard (2019 edition)[41], the traits of Konjac are identified as follows: Outer skin color: Tan or gray-brown. Cut face color:

White or yellowish-white. Surface texture: Granular protrusion and wavy wrinkles. Texture: Fragile and easily broken, with a rich powder-like texture. Sensation when chewed: A tingling sensation.

According to the Zhejiang Province Traditional Chinese Medicine Processing Standard (2015 edition)[42], the traits of Konjac are described as follows: Surface color: White, yellow to light brown. Surface texture: Relatively smooth, occasionally showing sunken stem marks, dotted root marks, and dark brown residual skin. Cut surface color: White to gray-brown. Cut surface texture: Fine granular protrusions or depressions, sometimes with a slight gelatinous luster. Texture: Hard. Odor: Slight fishy smell. Taste: Light taste. Sensation when chewed: A sense of tingling or numbness on the tongue.

According to the Shanghai Traditional Chinese Medicine decoction piece Processing Standard (2018 edition)[43], the traits of Konjac are described as follows: Surface color: Brownish yellow. Outer skin color: Grayish brown to tan brown. Visible marks: Stem marks and hemp beard marks may be present. Cut surface color: Yellowish white to pale brownish yellow. Cut surface texture: Granular protrusions. Texture: Fragile and easily broken. Texture description: Rich powder-like texture or slightly corneous (horn-like).

According to the Tianjin Traditional Chinese Medicine decoction pieces Processing Specification (2018 edition)[44], the traits of Konjac are described as follows: Cut surface color: Gray-white or pink. Presence of small vascular bundles: Most of the surface contains small vascular bundles. Surrounding color: Gray-brown. Texture: Wrinkled.

According to the Guangdong Provincial Traditional Chinese Medicine Standard (2011 edition)[45], the traits of Konjac are described as follows: Shape: The slices can be round, oval, or irregularly thick. Color: The slices are yellowish white or pale brown. Texture: The slices may be bent, and the edges can appear wrinkled. Cut surface color: Pale yellow-brown or yellowish-white with an uneven appearance. Odor: Slight gas-like smell. Taste: Slightly astringent taste.

2.2 Microscopic differentiation

Konjac and Konjac are both herbal plants, including species such as Konjac herb and willow[46], and their microscopic identification reveals numerous similarities. For instance, they share similarities in terms of the color of the posterior cortex and the presence of mucus cells containing calcium oxalate needle bundles, clusters, and thread-like ducts. However, there are also distinct differences between the two. Konjac has no secretion cavity but contains numerous starch grains and 6–7 rows of wood cells. On the other hand, Konjac features a large secretion cavity, polysaccharide particles, and 6–10 rows of wood cells. In the transverse section of Konjac, approximately 10 rows of wood-embolised cells form the brown cortex. It also exhibits a larger secretion cavity, calcium oxalate needle crystals, cluster crystals, and parenchyma cells containing starch granules. In Konjac powder, the parenchyma cells contain calcium oxalate cluster crystals, while calcium oxalate needle crystals are scattered or bundled within mucus cells. Thread-like or ring-like catheters, starch granules, and wood bolt cells can also be observed. The starch grains in Konjac are typically single and spherical, hemispherical, or round polygonal, occasionally forming complexes composed of 2–11 partitions[45].

2.3 Assay

The determination of KGM content can be carried out using various methods, including the phenol colorimetric method[47], Mannose weight method[48], Ferlin titration method[49], 3,5-dinitrosalicylic acid colorimetric method

(DNS method), gas chromatography (GC) and high-performance liquid chromatography (HPLC)[50]. In the study conducted by Yan Hua et al.[51], the DNS method was modified using non-toxic propranolol instead of phenol as the colorimetric agent. The results showed that the modified method still provided highly accurate measurements, suggesting its potential for wider application. Chen Jianfu et al.[52] employed single-factor and orthogonal tests to optimise the purification process of KGM, considering factors such as ethanol concentration, ultrasonic temperature, material-liquid ratio, ultrasonic power, and ultrasonic time. The optimized process achieved a high KGM content of up to 97.34%.

Chen Wentao et al.[53] extracted alkaloids from the plant using microwave-assisted extraction. The extracts were then qualitatively analysed using infrared spectroscopy. The extraction process was optimized, resulting in a yield of 0.216% under optimal conditions. Yang Xiaofan et al.[23] employed the Soxhlet extraction method to extract total alkaloids from the plant. Under the optimised process conditions, the yield of total alkaloids was determined to be 0.0939%. Long Deqing et al.[54–55] utilized the acid alcohol reflux and dipping method to extract total alkaloids from the plant. Their studies found that the yield of total alkaloids was 0.39% and 0.2%-0.28%, respectively. Sun Tianwei[20] employed the acid dye colorimetric method to measure the alkaloid content. Adding citrate-sodium citrate buffer with a pH of 5.4 made the detection process simpler, faster, and more sensitive.

Zhou Hao[30] extracted Ajjac ceramide using ethanol condensation reflux and performed one-factor experiments to determine the optimal extraction conditions. It was observed that different konjac species impacted the ceramide content, with the highest content of 43.75 μg/g among the samples with four different traits. Zhou Xiuxiu[30] calculated the amounts of ceramide in red konjac powder and skin samples as 0.36 mg/g and 0.48 mg/g, respectively. Liu Renping[56] investigated the effect of the reflux extraction method on the extraction of konjac ceramide and achieved an extraction amount of 4.34 mg/g under optimised conditions. The crude ceramide obtained was subjected to silica gel column chromatography using petroleum ether/ethyl acetate as the elution agent. After initial separation and purification, semi-prepared high-performance liquid phase separation was performed, obtaining a ceramide with a molecular mass 556. Zhang Weina[57] explored the influence of Soxhlet extraction on ceramide extraction using Konjac as the raw material. The results revealed that the extraction temperature was the main influencing factor, followed by ethanol concentration, extraction time, and liquid ratio. The final extraction rate obtained was 1.46%.

2.4 Other analytical methods

Willow et al.[58] conducted thin layer chromatographic identification of different batches of Konjac and Konjac. In TLC analysis, glucose and mannose were used as control substances. It was observed that Konjac showed spots at the corresponding positions of glucose and mannose, indicating the presence of both sugars. However, konjac only exhibited a spot at the D-glucose position. The TLC unfolding conditions employed were trichloromethane-methanol (9.5–0.5). It was found that Konjac exhibited more thin layer spots compared to konjac, suggesting a greater diversity of components in Konjac. On the other hand, the spots observed in Konjac were relatively single, indicating a lower variety of components present.

Ceramide, being weak or lacking a chromophore, poses challenges in its detection using UV spectroscopy. Therefore, derivatization techniques are employed to enhance its detectability. Ding Yaping[33] utilized benzoyl chloride to derivatise ceramide in konjac powder. The presence of ceramide in konjac powder was confirmed within a certain error range, and its concentration was determined to be 0.047% by HPLC analysis. Zhu Lian

Lian[32] employed an alizarin-ethanol color developer in thin layer chromatography inspection. A distinct color band was observed in the middle of the thin layer plate, suggesting the likely presence of ceramide in konjac fly powder.

In the study by Zhou Qiwu[19], ICP-MS was employed to detect six heavy metal elements, namely Cr, Ni, Cd, Pb, As, and Hg, in the samples. The results indicated that the levels of these elements were within the food safety limit standards set by China.

Current status of quality standards

Despite its long history of use, *Amorphophallus konjac* does not have a unified national quality standard as of the Chinese Pharmacopoeia editions of 2020 and 2015. However, regional quality standards have been established in provinces such as Anhui, Zhejiang, and Shanghai. A comparison of the current domestic quality standards for Snake Six Valley is presented in Table 1.

The medicinal substrate of *Amorphophallus konjac* is subjected to varying local standards, and there is a diverse range of mixed-use observed in the Chinese medicinal materials market. In the case of compound formulations, the large dosage, toxicity, and lack of corresponding exclusive examination pose challenges in evaluating and controlling the quality of finished products. The existing quality control standards for *Amorphophallus konjac* primarily consist of local standards from different provinces and cities, focusing mainly on trait identification, such as microscopic identification. However, these standards lack exclusive testing items, including physical and chemical identification and thin layer identification, making it challenging for relevant departments to detect and control the quality of *Amorphophallus konjac* effectively. Therefore, it is crucial to establish exclusive identification items specific to *Amorphophallus konjac*.

Table 1
Local quality standards for *Amorphophallus konjac*

Standard	Name	Source	Differentiation		Check up /%		Assaying
			Shape and properties	Microscopic differentiation	Shuifen	Total ash	
Anhui Provincial Specification for Processing of Chinese Medicine (2019 edition)	*Amorphophallus konjac*	The dry tubers of the thin-hairy Konjac	√	×	≤ 13.0	≤ 8.5	×
Shanghai Code for Processing of Chinese Medicine (2018 Edition)	*Amorphophallus konjac* / Konjac	The dry tuber of konjac or konjac	√	×	×	×	×
Processing Specification for Tianjin (2018 edition)	*Amorphophallus konjac*	Konjac dry tuber	√	×	×	×	×
Zhejiang Provincial Code for TCM Processing (2015 edition)	*Amorphophallus konjac* / konjac	The dried tubers of konjac or Huadong konjac	√	√	×	×	×
Guangdong Provincial Traditional Chinese Medicine Standard (2011 edition)	Konjac / Single tallow	The dried tubers of the konjac	√	×	≤ 15.0	≤ 7.0	×
Hubei Province Traditional Chinese Medicine Standard (2009 edition)	Devils tongue	The dried tubers of the konjac	√	√	×	×	×

Challenges in quality control of Amorphophallus konjac

4.1 Lack of identification items

The original medicinal plants of *Amorphophallus konjac* share significant similarities in appearance and morphology, making it challenging to distinguish them solely based on these characteristics. Moreover, the ingredients present in these plants are complex, further complicating their identification. The lack of sufficient research reports and the absence of landmark ingredients for identification exacerbate the difficulties in identifying traditional Chinese medicines like *Amorphophallus konjac*. There is a notable shortage of identification items, with most standards focusing on trait identification. Micro differentiation and thin layer identification are rarely mentioned or utilized in the identification process. In order to address the challenges posed by confused products and uneven quality in Snake Six Valley, relying solely on trait identification is insufficient. It is crucial to incorporate additional methods such as spectrum analysis, chromatography, and other advanced technologies to enhance the authenticity evaluation of *Amorphophallus konjac*. New technologies such as DNA barcoding for molecular identification and TCM fingerprint spectrum analysis can provide more accurate and reliable identification results. Furthermore, it is important to continually update and refine the existing identification items to keep pace with scientific advancements and address emerging challenges.

DNA barcoding molecular identification is a powerful technique in molecular biology for species identification. It utilizes a short and conserved DNA sequence in the genome to supplement traditional morphological identification methods, providing effective means of identification[59]. In the case of *Amorphophallus konjac*, Willow et al. [60] utilized the ITS/ITS 2 sequence for identification and analysis. However, it is worth noting that there are challenges in extracting DNA from the slices of Snake Six Valley. The presence of polysaccharides and other components, particularly the unique physicochemical properties of glucomannan, can hinder the DNA extraction process. Therefore, further research is necessary to develop appropriate extraction methods to overcome these challenges and enable successful DNA extraction from Snake Six Valley slices.

4.2 Improvement of inspection items

Ensuring the purity of medicinal materials through inspection is crucial for maintaining medication safety. With the increasing concerns about environmental degradation, soil pollution, heavy metals, and pesticide residues, it has become imperative to include comprehensive inspection items in quality control standards. Improper transportation and storage conditions can lead to mold growth and material deterioration. Table 1 reveals the current processing specifications for TCM decoction pieces in Anhui Province (2019 edition)[41] and Guangdong Province (2011 edition)[45]. These specifications include limited standards for "water" and "total ash content", such as 13% and 8.5% in Anhui Province and 15.0% moisture and 7.0% total ash score in Guangdong Province. These standards benefit medicinal materials' transportation, storage, and therapeutic efficacy. However, it is important to note that many other local quality standards lack inspection items, such as moisture content, total ash, toxic ingredients, heavy metals, harmful elements, and pesticide residues. The absence of these inspection items hampers the ability to ensure the safety of *Amorphophallus konjac* as a medicinal product. Therefore, incorporating these inspection items is necessary to improve and supplement the existing quality control standards. If deemed necessary, the safety of medicinal material samples can be evaluated through comprehensive assessments to identify potential issues. This can involve selecting identification items that possess high specificity and sensitivity.

4.3 Supplementation of extract measurement

The tumor inhibition effect of *Amorphophallus konjac* is significant, although identifying the same active component and accurately measuring its content is challenging. *Amorphophallus konjac* is typically used in decoction form, following the formulation of quality standards based on other Chinese medicinal materials. According to Lu Yun et al. [61], establishing a quality standard for *Amorphophallus konjac* involves determining the percentage of extract and the total peak area of characteristic peaks. By studying the decoction of *Amorphophallus konjac*, specific standards for alcohol-soluble and water-soluble extracts can be developed accordingly. Currently, the Guangdong Province of Traditional Chinese Medicine standard[45] is the only one that specifies that the water-soluble Konjac extract should be less than 19.0%. The determination of extract content plays a crucial role in assessing the authenticity and quality of traditional Chinese medicine and is a routine item in quality control. Increasing the measurement control standard for extracts makes it possible to effectively identify component loss that may occur during the processing, transportation, and storage of Snake Valley. This measure aims to minimize the circulation and use of inferior Snake Valley in the market and ensure the integrity of its quality.

4.4 Optimisation of assay item

According to the current quality standard of *Amorphophallus konjac*, content determination items are deficient, and the absence of clear index components hinders the comprehensive evaluation of the quality of decoction pieces. Therefore, it is crucial to establish index components or multi-index content to enhance the content items and improve the specificity of detection. Glucomannan, ceramide, alkaloids, and other significant chemical components in *Amorphophallus konjac* require the development of relevant standards for their investigation. In the future, glucomannan in *Amorphophallus konjac* can be determined using solvent and microwave-assisted extraction methods, which can contribute to improving quality control measures.

4.5 The application of fingerprint analysis

Fingerprint mapping technology in traditional Chinese medicine (TCM) involves obtaining a particular drug's spectrum or chromatographic profile. It allows for accurate identification and characterization of various components present in the TCM, similar to a human fingerprint with unique characteristics. This technology has evolved with modern science and technology advancements, leading to various TCM fingerprints. These fingerprints can encompass TCM biology, metabolism, spectroscopy, and chromatography. Chinese medicine fingerprint mapping technology has proven valuable in various aspects, such as identification, composition analysis, and quality control. It serves as a reference for establishing quality standards and promoting overall quality improvement in the field of TCM. The TCM fingerprint map aligns with the unique characteristics of TCM, characterized by complexity and ambiguity. As research on TCM fingerprint mapping continues, quality standards can be optimised to monitor the quality of fingerprint maps or characteristic profiles effectively. These fingerprint maps provide crucial information about the active ingredients present in TCM, allowing for enhanced quality control measures.

Conclusion

Amorphophallus konjac has been used for medicinal purposes throughout its long history, including treating cough, stagnation, carbuncle, snake bites, and even as a potential anti-cancer agent. However, the active components, toxic components, and precise anti-cancer mechanisms of *Amorphophallus konjac* are not yet fully understood, and the available literature on these aspects is limited. In traditional Chinese medicine (TCM), herbal medicine is significant as it is vital in treating and preventing diseases. The stability of the decoction pieces is crucial as it directly impacts the quality of Chinese patent medicine and subsequently affects the effectiveness and safety of clinical treatments. The absence of index components for qualitative and quantitative analysis in the study of the quality standard of *Amorphophallus konjac* herbs poses challenges in fully and accurately assessing the quality of the medicinal materials. Therefore, to ensure the quality and safety of *Amorphophallus konjac*, it is essential to optimise their quality standards. There is a lack of unified national standards, and existing local standards vary. The situation calls for comprehensive and standardized guidelines to be established. *Amorphophallus konjac* is widely used for various purposes, such as cancer treatment, weight control, and anti-fatty liver. However, the qualitative and quantitative analysis methods need improvement to meet modern safety requirements. Therefore, further research and improvement of quality standards are necessary to ensure the high quality and reliability of *Amorphophallus konjac*, providing patients with safe and effective medications.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors Contribution

LJY conceived the research idea. HTT conducted the experiments, collected plant specimens, analyzed and interpreted the data in addition to preparing the first draft and they critically read and revised the paper. All authors read and approved the final manuscript.

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Figures

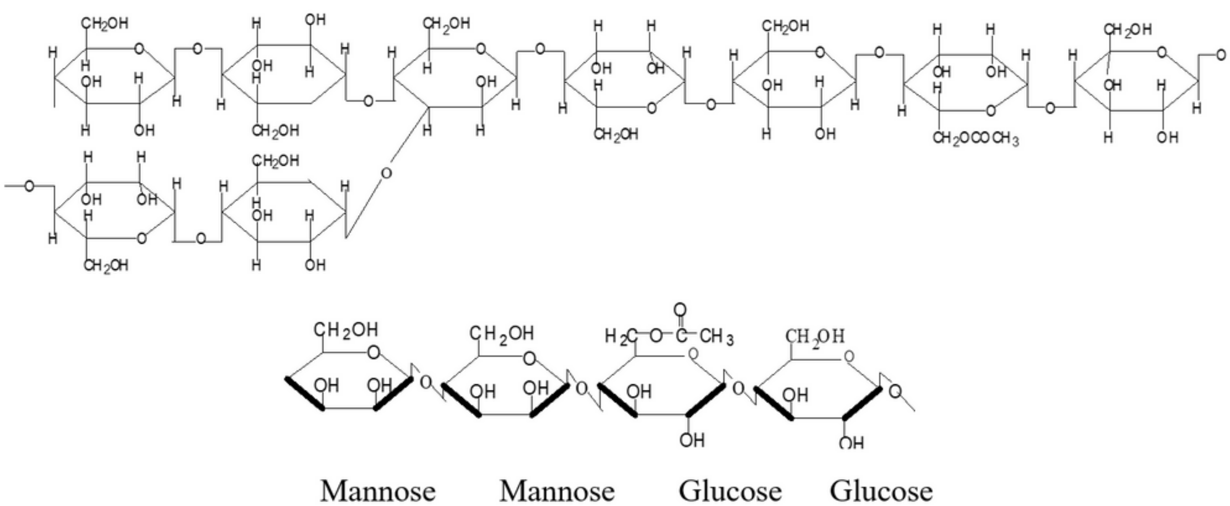


Figure 1

Schematic representation of the basic structure of glucomannan

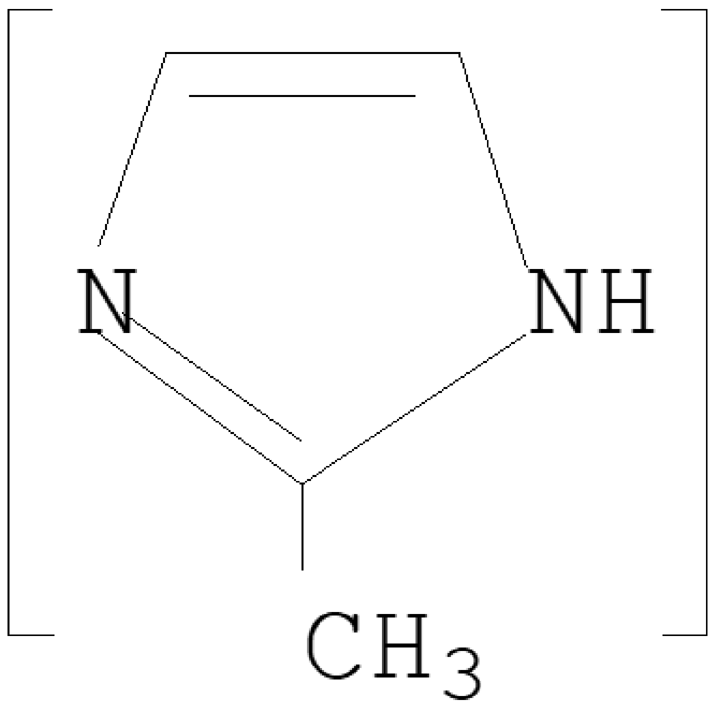


Figure 2

Structure of 5-Methylimidazole

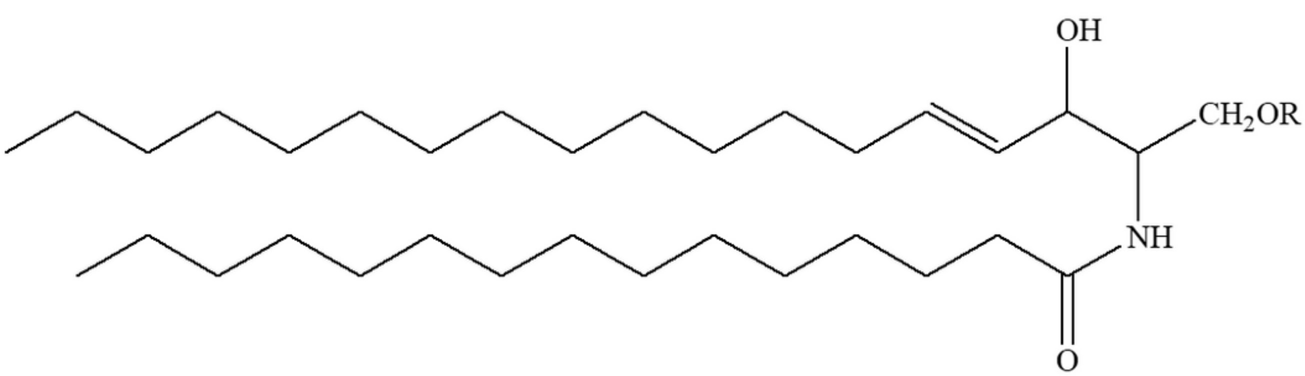
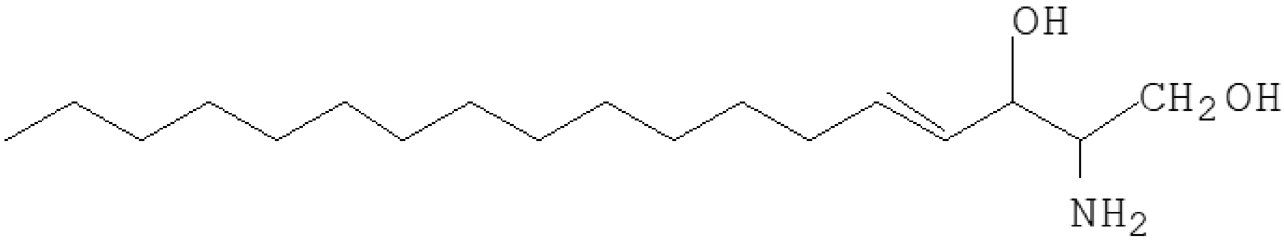
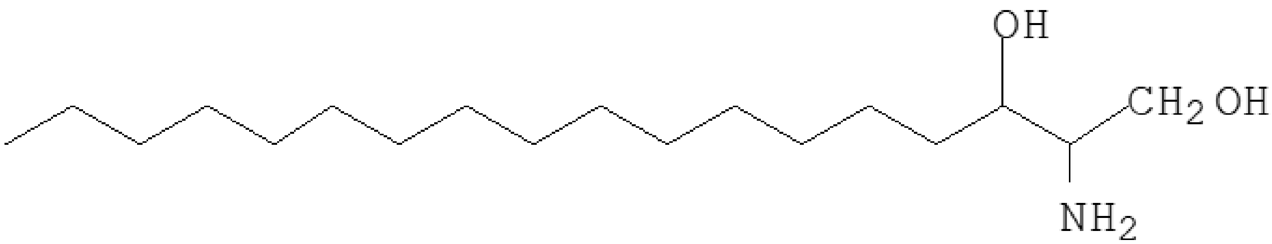


Figure 3

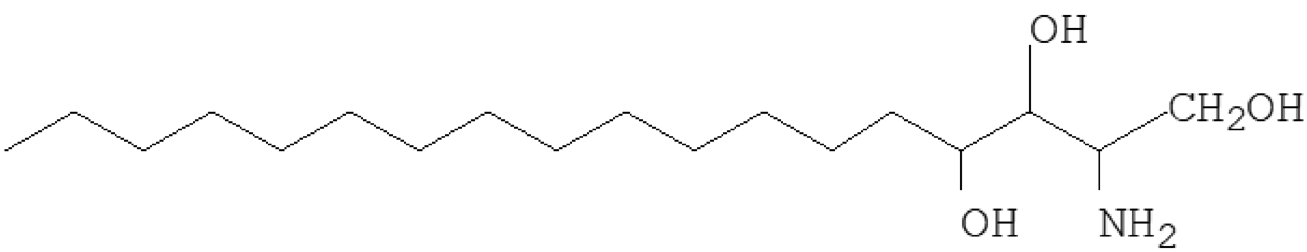
Basic structure of the ceramide



Sphingosine (SPH)



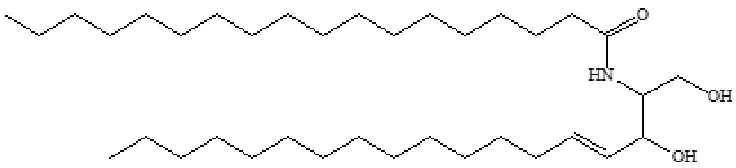
Dihydrosphingosine (DHS)



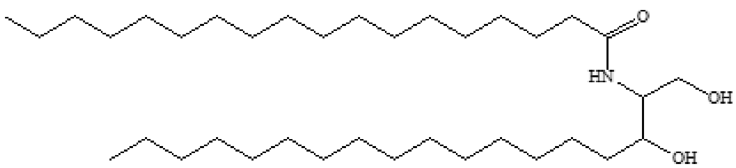
Phytosphingosine (PHS)

Figure 4

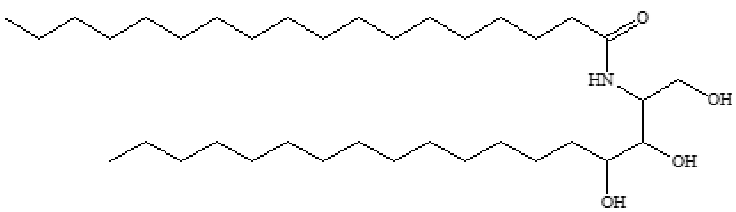
Structure of the three sphingosines



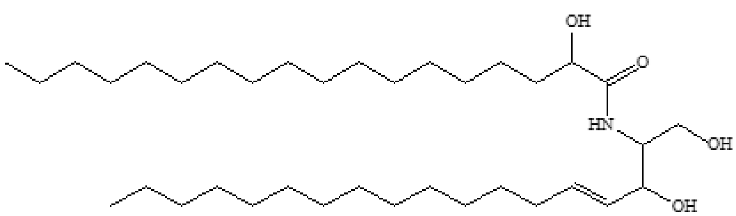
Ceramide I



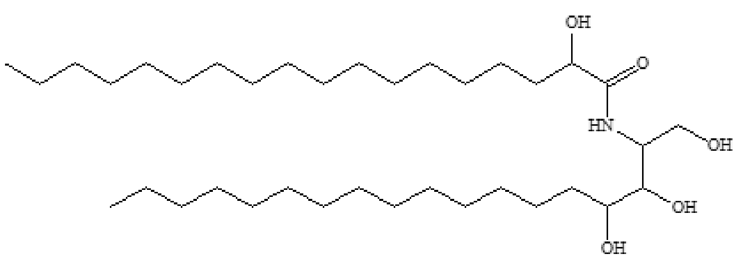
Ceramide II



Ceramide III



Ceramide IV



Ceramide V

Figure 5

Structures of several common ceramides

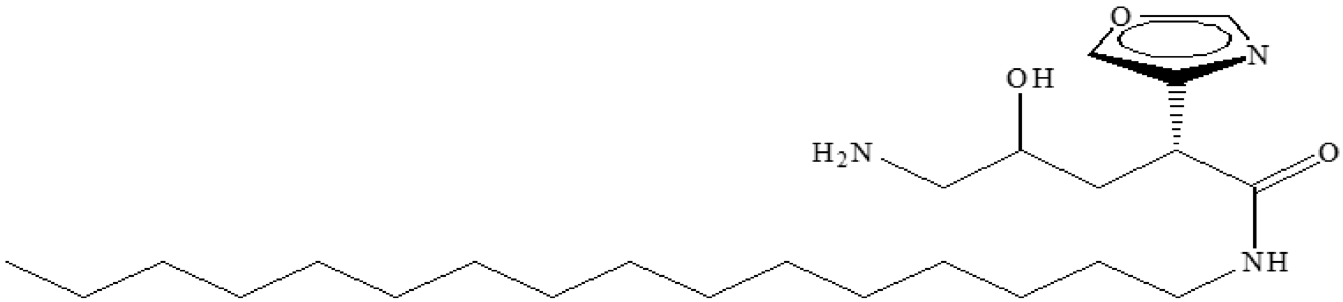


Figure 6

Molecular structure of konjac ceramide

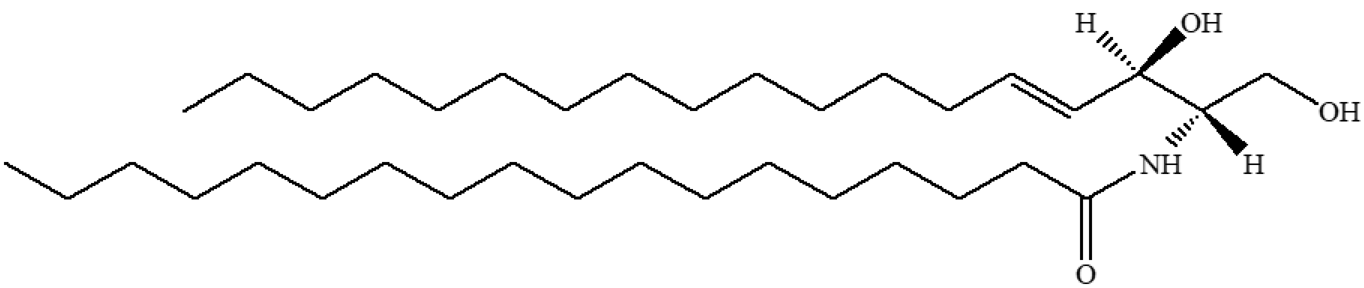


Figure 7

Structure of ceramide

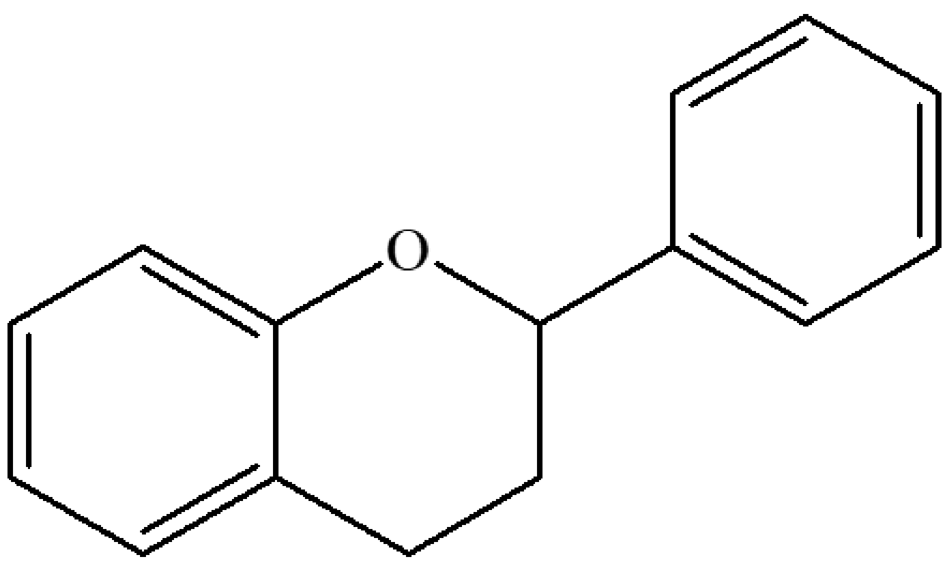


Figure 8

Plot of the basic parent nuclear structure of flavonoids